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BIOLOGY OF *RHYNENCINA LONGIROSTRIS* JOHNSON (DIPTERA: TEPHRITIDAE)

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Abstract.—Adults of *Rhynencina longirostris* Johnson were successfully reared from larvae in the achenes of *Smallanthus uvedalius* (L.) Mackenzie (Asteraceae), the first confirmed host plant record for this fly. Adults of *R. longirostris* are univoltine with a flight-time corresponding to the flowering period of *S. uvedalius*, which is July–September in the Great Smoky Mountains National Park. *Heteroschema* sp. (Hymenoptera: Pteromalidae) parasitoids and *Homoeosoma* sp. (Lepidoptera: Pyralidae) feeding in capitula of the host are probably significant sources of mortality of *R. longirostris* immature stages.

Key Words: *Smallanthus uvedalius*, Great Smoky Mountains National Park, All Taxa Biological Inventory, host plant

In connection with the All Taxon Biological Inventory (ATBI) that was initiated in the Great Smoky Mountains National Park (GSMNP) in 1997, Gary Steck (GJS) and Bruce D. Sutton (BDS) joined a dipterists' 'collecting blitz' in late May, 1999. During this visit, GSMNP collections curator, Don DeFoe (DD), kindly allowed us to peruse the collection in the Natural History Museum (GSNP) at the park headquarters at Sugarlands, where we noted the presence of six specimens of a rarely collected tephritid fly, *Rhynencina longirostris* Johnson. These specimens are the first known record of *R. longirostris* in the state of Tennessee (Steck and Sutton 2000), with label data as follows: "Tennessee: Sevier Co., Park HQ, 1520' elev., 5 Aug 1996." DD, as collector of the specimens, was able to take us directly to the location and the plants upon which the adult flies had been taken; this was a small patch of *Smallanthus uvedalius* (L.) Mackenzie (Asteraceae) in a disturbed, shaded area next to a parking lot. The flies had been on the flower heads at the time of capture. That discovery prompted all of the following investigations reported here.

Rhynencina is a small genus comprising only five New World species: three species are present in South America, one in Mesoamerica, and one, *R. longirostris*, in eastern North America (Freidberg and Norrbom 1999). The latter has been recorded from Pennsylvania, Maryland, North Carolina, and Georgia (Foote et al. 1993), and Tennessee (Steck and Sutton 2000). Limited host plant information is available for the genus. *Rhynencina emphanes* (Steyskal) (Colombia) has been reared from flowers of *Espeletia* sp.; adults of *Rhynencina dysphanes* (Steyskal) (Andean countries), *Rhynencina spilogaster* (Steyskal) (Mexico, Central America), and *R. longirostris* have all been collected on *Polymnia* sp. and/or the closely related genus *Smallanthus*; and *R. spilogaster* has been observed ovipositing

into flowers of *Smallanthus* sp. (Freidberg and Norrbom 1999) (host genera are all of Asteraceae, tribe Heliantheae); finally, *R. longirostris* was reared for the first time from *Smallanthus uvedalius* (Steck and Sutton 2000) as more fully described below.

Smallanthus uvedalius (formerly *Polymnia uvedalia*), commonly known as leafcup or bearsfoot, is an herbaceous perennial that is widespread in woods and meadows of the eastern United States and flowers from summer to fall. Disk flowers are sterile, and thick achenes are produced from the ray flowers (Cronquist 1980).

Materials and Methods

Most data were gathered from two patches of the host plant, *Smallanthus uvedalius*, in the GSMNP, one at Metcalf Bottoms and another at Sugarlands. The former stand of plants was spread along about 250 m of roadside and contained an estimated 1,300–1,800 plants. The patch at Sugarlands was about 20 m × 3 m with an estimated 150–200 plants.

Data on immature stages was obtained by dissections of flower and seed heads that were collected from 1999 to 2001. On 24 and 27 September 1999, DD collected mature seed heads from Metcalf Bottoms and Sugarlands, respectively, packaged them in brown paper bags, and sent them by overnight delivery to Gainesville, Florida; two additional collections of dried seed heads, from Sugarlands on 5 October, and from Metcalf Bottoms on 13 October 1999, were also sent to Gainesville in the same manner. In total about 50 dried seed heads were collected in 1999. Dissection of these heads revealed the presence of likely tephritid larvae inside achenes, as described more fully below. In an attempt to rear adults, several 100s of unopened achenes were held dry and at room temperature in the laboratory until mid-November, 1999. At that time they were placed on damp sphagnum moss in a loose-lidded plastic container and kept in a refrigerator at about 4°C to break diapause. In mid-April, 2000, the achenes in their bed of damp sphagnum moss were transferred to a glass terrarium with screened top and bottom. At this time, some of the achenes were observed to be completely softened and disintegrating, while others were still very hard as before. Thereafter the terrarium was kept in a shaded area outdoors under ambient Gainesville conditions (likely much warmer and drier than Smoky Mountains conditions), or alternatively under fluorescent lighting in a garage utility room with more moderate temperature and humidity conditions. The terrarium was watered sporadically.

Further dissections were made from seed heads collected from Metcalf Bottoms and Sugarlands 4–8 September 2000 (Lionel Stange); from buds and flowering heads collected on 13 July 2001, Metcalf Bottoms (GJS, Victor M. Steck); and from buds, flower heads, and seed heads collected 2–4 September 2001 (GJS, BDS), Metcalf Bottoms.

Observation and collection of adult stages were made during 12–13 July 2000 (GJS, BDS), 2 and 13 July 2001 (GJS, VMS), and 2–4 September 2001 (GJS, BDS).

All life stages of *R. longirostris* are vouchered at the Museum of Entomology, Florida State Collection of Arthropods (FSCA) in Gainesville. A full description of all immature stages will be published at a later date.

Results

Rearing to adult stage.—Seeds held for overwintering in 1999 began germinating in mid-May, 2000. In mid-July the terrarium was brought back into the laboratory in Gainesville for closer observation, and between 24 and 27 July 2000 three adult *R. longirostris* flies emerged, thus proving *S. uvedalius* to be a host of the immature stages. On 12 July 2000, GJS and BDS carefully combed the litter below the host plants at Sugarlands looking for remains of the previous year's achenes. A few dozen intact achenes were located (along with numerous

fragments) and held for rearing, but nothing came of them.

Immature stages in flower and seed heads.—On 28 September 1999, GJS and BDS dissected and examined the contents of some of the seed heads collected by DD on September 24 and 27 at Metcalf Bottoms and Sugarlands, respectively. There were no exposed tephritid immature stages present in the heads. Most seed heads retained a peripheral ring of black, dry, and well-filled achenes (disk flowers are sterile, and a maximum of 11–12 seeds per head develop from the peripheral ray flowers). Achenes were very hard, and only with considerable difficulty did we manage to open them to examine the contents. Some achenes, often slightly lighter-colored and smaller, contained a bright yellow larva. In each case, a single larva filled the seed cavity, with only the thin, but hard, pericarp surrounding it. A total of seven 3rd instar larvae (based on mouthhook size; body lengths varied from about 1.6 to 3.7 mm) and five puparia was extracted in this fashion. Two of the puparia were held dry in a vial; in May 2001, it was noted that two adults of a *Heteroschema* sp. (Hymenoptera: Pteromalidae) had emerged. In addition, of the three puparia preserved in alcohol, two clearly contained hymenopteran larvae. Thus, the latter are likely larval-pupal parasitoids. There were no signs of exit holes or 'windows' prepared by the *R. longirostris* larvae for later escape from the achene. The larvae were preserved in 70% alcohol after killing in hot water. Numerous seeds contained frass and tiny Lepidoptera larvae. There were also a few small, non-tephritid Diptera pupae in the dry seed heads, but not in the achenes.

From the Sugarlands (5 October 1999) and Metcalf Bottoms (13 October 1999) seed head collections, we attempted to get a more quantitative estimate of their contents. To avoid destroying the achene contents, a small hand-held drill with a fine, bur bit was used to grind away enough of the pericarp to determine the contents. Of 221 achenes examined in this manner, a total of 11 live *R. longirostris* 3rd instars, one dead 2nd instar, and 12 live pupae was obtained. The larvae had consumed the seed only. Two of the puparia were held dry for emergence—one yielded an adult *Heteroschema* sp., and the other an unemerged adult *R. longirostris*; five of the other ten puparia clearly contained hymenopteran larvae. Thus, this limited sample indicates a parasitism rate of 50%. Of the remaining achenes, 102 contained seemingly viable seeds (46%) and 89 appeared non-viable (empty or shriveled) (40%); another six achenes were full of insect frass (3%). The 221 achenes examined were not selected randomly, as we were trying to maximize the number *R. longirostris* specimens found, and so chose smaller, discolored achenes; thus, these numbers do not accurately reflect actual infestation rates. See Table 1 for a summary of achene contents.

Because of the time, effort, and difficulty encountered in dissecting dried achenes, the seed heads collected in year 2000 were treated differently: achenes were separated from their capitula, placed into a beaker of water, and gradually heated to about 70°C; after cooling they were transferred to 70% isopropanol. After a few months of storage in alcohol, the achenes were much more easily dissected with forceps without damaging their contents. One hundred achenes from each site were randomly selected and dissected. From the Metcalf Bottoms collection, these yielded 24 immature specimens of *R. longirostris* (three 1st instars, seven 2nd instars, four 3rd instars, ten pupae; nine of the ten puparia clearly contained a hymenopteran larva) (Table 1). As noted above, only a single *R. longirostris* larva or puparium was present in a given achene. Each of the immature specimens, excepting one of the 1st instars, was inside the seed coat. In the case of the 1st and 2nd instars, the surrounding seed coat was merely a shrivelled bag. (These could not be distinguished from the seed coat of numerous other inviable but uninfested seeds.)

Table 1. Immature stages of *Rhynencina longirostris* in seed heads of *Smallanthus uvedalius*.

Contents	Oct 1999	Sept 2000	July 2001	Sept 2001
No. achenes examined	221	100	237	ca. 117
No. eggs	0	0	46	0
No. 1st instars	0	4	0	2
No. 2nd instars	1	7	0	1
No. 3rd instars	11	4	0	0
No. puparia	12	10	0	0
% parasitized puparia	50%	90%	—	—
Lepidoptera frass	+	11*	0	ca. 70

+ Indicates present, but not quantified.

* Does not represent total damage from Lepidoptera feeding, because only intact achenes were selected for dissection.

There was nothing resembling an obvious oviposition scar on the achene exterior, suggesting that oviposition occurs directly into the flower ovary at a very early stage of development. Of the 100 achenes dissected, only 32 contained what appeared to be viable seeds. Of the remainder, 11 had been destroyed by Lepidoptera larvae as evidenced by their frass; other seed coats were empty or only partially filled for no apparent reason. Infested achenes were indeed smaller than those with viable seeds. Average diameter of infested achenes was 4.7 mm (range 3.3–5.4 mm, N = 24), while that of achenes with viable seed was 5.1 mm (range 4.2–6.0 mm, N = 32). Infestation at the Sugarlands site was very low: of 100 achenes, only two contained *R. longirostris* (one 1st instar and one 2nd instar), while 71 contained apparently viable seeds.

Plant collections made in year 2001 were treated somewhat differently from before. Entire capitula (rather than culled, intact achenes) were subjected to hot water treatment, then preserved in alcohol. This provided data on *R. longirostris* egg deposition and the full impact of Lepidoptera larvae on seed production.

On 2 July 2001 there were only a very few buds of *S. uvedalius* to be found at Metcalf Bottoms, and none was larger than about 5 mm in diameter. By 13 July, a small number of buds had developed to the stage where capitula disks were exposed and petals of ray flowers partially developed. All of the capitula (88 total) in the most advanced stage of development were collected, preserved in alcohol, and examined under a microscope (see Table 1). Oviposition and probe punctures, where an aculeus had penetrated the outer phyllaries and young achenes, were easily visible. Dissected heads ranged in size from unopened buds as small as 4 mm diameter to the most developed capitulum in the population, which had a 12 mm-diameter receptacle (measured with outer phyllaries removed) and petals of 12 mm length. Six capitula (7%) were found with eggs oviposited into achenes as follows: (1) two of ten achenes with one egg each in a 12 mm-diameter capitulum with petals expanded, which was the most developed capitulum present at Metcalf Bottoms; (2) nine of nine achenes with a range of two to five eggs per achene in a 10 mm-diameter capitulum with outer phyllaries reflexed and one petal just beginning to unfurl; (3) five of 11 achenes with one egg each in a 10 mm-diameter capitulum with none of the petals unfurled; (4) four of nine achenes with one to two eggs each in a 10 mm-diameter capitulum with bracts partly reflexed and no petals; (5) four of 11 achenes with one egg each in a 10 mm-diameter capitulum with none of the petals unfurled; and, (6) one of 12 achenes with one egg in a 9 mm-diameter capitulum with disk exposed and no petals. The achenes containing eggs ranged from 1.2 to 2.8 mm long. Two capitula of 9 mm di-

ameter had punctures that indicated probing by an aculeus but no eggs. There were no probing punctures found on achenes of unopened buds or heads with a receptacle diameter of less than 9 mm, although a few probing punctures were found in the phyllaries of smaller capitula (two 8-mm and one 6-mm capitula). None of the eggs had yet hatched. Thus, the few days before 13 July at the time when capitula of *S. uvedalius* first began to open and attained about 9 mm diameter, marked the very beginning of the reproductive period of *R. longirostris* at Metcalf Bottoms for the year 2001.

By 2 September 2001, the *S. uvedalius* population at Metcalf Bottoms was found to be highly senescent, i.e., fewer than 1% of all flower heads remained in bud or the flowering stage. No adult flies were observed at this time, but oviposition had occurred probably not long before, as 1st instar larvae were still to be found in developing achenes (Table 1). A notable observation at this time was the very extensive feeding damage by larvae of a *Homoeosoma* sp. (Lepidoptera: Pyralidae). Of the 14 capitula examined, 11 had been fed upon by *Homoeosoma*; seven were 90–100% consumed, and three were 33–50% consumed. Thus, it is likely that some, perhaps many, late season *R. longirostris* immature stages were eaten by larvae of *Homoeosoma* sp.

Observations on adult flies.—During 12–13 July 2000, GJS and BDS found male and female adult *R. longirostris* in abundance on *S. uvedalius* foliage and flower heads at the Metcalf Bottoms site. Plants were well developed at this site, many to 1 m or more in height, and some were in flower. A smaller number of adult *R. longirostris* was observed at Abram's Creek campground, also on *S. uvedalius*, but no adults were found at the Sugarlands site, where *S. uvedalius* was not yet in flower.

On 2 July 2001, very few or no flower buds were present at Metcalf Bottoms and Sugarlands, respectively, and no adults were observed. On 13 July 2001, one female and three males were observed at Metcalf Bottoms. One male was observed mounted on the female on the most developed flower head at this site, and a second male was facing them on the periphery of the same flower head. Therefore, courtship and mating apparently occur on the host plant, as is typical for many tephritid flies. By 2 September 2001, no adults were to be found at Metcalf Bottoms, where flowering of host plants was nearly finished. However, in other areas, such as Cades Cove and Abrams Creek campground, many plants were still in full flower, and adult *R. longirostris* were collected at Abrams Creek campground on 4 September 2001.

Discussion

Based on our rearings and collections from 1996 to 2001, we conclude that *R. longirostris* is univoltine with a flight-time corresponding to the flowering period of *S. uvedalius*, i.e., July–September in the GSMNP. Local populations of *S. uvedalius* flower at somewhat different times. It remains to be determined whether the emergence and disappearance of the associated fly populations are locally synchronized with their hosts, or whether flies disperse among local host populations according to the presence/absence of capitula at an appropriate stage for oviposition. Courtship and mating occur on the host plant. Oviposition probably begins as soon as achenes of *S. uvedalius* develop to the minimum size needed to support larval development. At the beginning of the flowering season, when buds are scarce, a single achene may have several eggs oviposited; thereafter, females lay only a single egg into an achene. Only one larva develops per achene. Parasitism by hymenopteran larval-pupal parasites may be very high. Given the extreme hardness of the mature, dry achenes, hymenopteran parasites may be able to attack only during the early stages of achene development. Late in the flowering season, Lepidoptera larvae feed voraciously on capitula and probably also inflict significant

mortality on immature stages of *R. longirostris*. Many achenes from the preceding year's seed crop that were found under host plants at Sugarlands appeared to have been cracked open leaving only very hard fragments. It is possible that vertebrate seed predation is another significant cause of mortality, particularly if rodents, such as squirrels or mice, or seed-feeding birds, are targeting the slightly more easily opened, protein-rich, infested achenes. By what means the adult flies escape the achene remains unknown. Dried achenes are very hard and it is difficult to imagine an adult extricating itself without a pre-thinned 'window' in place. Perhaps they rely on simple weathering from the elements to decompose the pericarp to a point where adults can eclose and escape successfully.

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